3372-Pos Board B233
Physiological Magnesium Influx Pathways in Cardiac Myocytes
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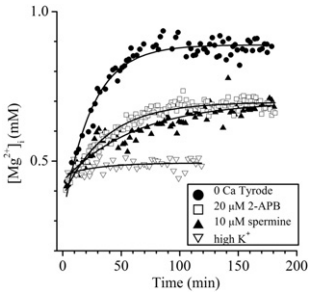
Rat ventricular myocytes were loaded with a fluorescent Mg^{2+} indicator furat-pra (mag-fura-2), and intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) was measured. Incubation of the cells in a Mg^{2+}-free Ca^{2+}-free high-K^+ solution for 20 min caused a decrease in $[Mg^{2+}]_i$ from ~0.9 mM to 0.2-0.5 mM (Mg^{2+} depletion). When the Mg^{2+}-depleted cells were then perfused with Ca^{2+}-free Tyrode's solution that contained normal levels of Na^+, K^+ and Mg^{2+}, $[Mg^{2+}]_i$ gradually returned back to the basal level. The time course of the $[Mg^{2+}]_i$ recovery was well fitted by a single exponential function with an average time constant of 42 min (25°C). We analyzed the first derivative of the fitted curve at time 0 (initial $d[Mg^{2+}]_i/dt$) as an initial rate of Mg^{2+} influx. The initial $d[Mg^{2+}]_i/dt$ was, on average, 0.27 ± 0.04 μM/s, and was unchanged by the presence of 1 mM extracellular Ca^{2+} (0.23 ± 0.03 μM/s). Membrane depolarization by high K^+ significantly decreased the rate to 0.053 ± 0.02 μM/s. The initial $d[Mg^{2+}]_i/dt$ was also significantly reduced by 20 μM 2-APB (0.13 ± 0.04 μM/s) or 10 μM spermine (0.14 ± 0.02 μM/s), known inhibitors of TRPM7/MIC channels. The results suggest that TRPM7/MIC channels serve as a physiological influx pathway for Mg^{2+} in cardiac myocytes.



3373-Pos Board B234
Mechanistic Insights into the Twin-Arginine Translocation Cycle of Escherichia Coli by an In Vivo Single-Molecule Approach
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The twin-arginine translocation (Tat) system is a unique protein targeting pathway which has been found in bacteria, archea and chloroplasts as well as in plant mitochondria. Its ability to transport fully folded proteins across the cytoplasmic membrane distinguishes it from other translocation pathways. In E. coli the essential components of the system, TatA, TatB, and TatC, have been isolated in complexes of different size, suggesting that a fully active Tat complex forms only transiently. Furthermore, the precise steps in the translocation cycle remain mostly unknown.
We perform fluorescence microscopy and single-particle tracking to gain deeper insight into the dynamics of the Tat machinery. To this end, bacteria expressing low levels of GFP-fused Tat subunits are imaged with sensitive laser-illuminated wide-field fluorescence microscopy. Mobile fluorescent spots are observed, and their intensity and location determined by fitting them with a 2D Gaussian function. The trajectories of these spots are then established by linking the Gaussian fits in successive frames.
Our data shows that diffusion of TatA-eGFP is heterogeneous, and that its average diffusion coefficient decreases when translocation substrate is overexpressed. Moreover, TatA-eGFP mobility depends on the existence of an electrochemical potential, which is the driving force behind the Tat translocation system. This could suggest that TatA-eGFP complexes undergo a topological transition upon becoming 'translocation active'.

Membrane Receptors & Signal Transduction II

3374-Pos Board B235
Model Liquid Droplets to Explore T-Cell Surface Dynamics upon Activation
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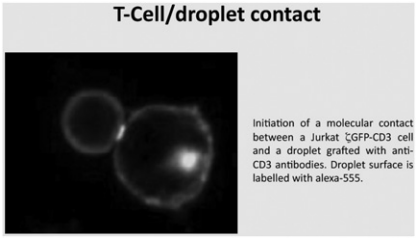
The T-cell is a central player of the immune response in mammals. It recognizes antigen on the surface of dedicated cells through the formation of several pairs of receptors and ligands in a micrometric bi-dimensional intercellular contact leading to cell activation. In order to simplify and dissect this process, we have developed model colloids grafted with defined ligands.
Here, we will show T-cell interaction with liquid colloids grafted with anti-CD3 targeted to the T-cell receptor (TCR) CD3ε subunit. Using fluorescence confocal microscopy, and ζCD3-GFP-expressing Jurkat cells, we have evidenced the active TCR-CD3 migration induced by anti-CD3 grafted droplets and the remarkable ligand dynamics induced by the cell contact on the droplet side. Cell activation was followed in parallel monitoring the intracellular calcium. Eventually, we have compared the dynamical properties of this cell-droplet contact with those of the contact driven by passive physico-chemical properties between two complementary droplets. Similarities between these two systems were limited to the initiation step of the process.
We concluded that the cell-droplet contact, conceived here as a simplified immune synapse, resulted from active cell mechanics and not from spontaneous self-assembly processes.



3375-Pos Board B236
The Molecular Basis for Lipid Recognition and Signaling in the Toll-Like Receptor Complex and a Family of Lipid-Binding Proteins
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The transmembrane toll-like receptors (TLRs) are the initial gateway to almost all mammalian inflammatory responses to invading microbes. They are specialized for binding ligands with diverse structural and physiochemical properties ranging from microbial cell wall components to nucleic acids, and there is substantial interest in their pharmacological manipulation. TLR4 binds specifically to lipopolysaccharide (LPS), the central glycolipid component of Gram-negative bacterial outer membranes. Minute amounts of lipid A, the bioactive component of LPS, are an early sign of infection, and the association of TLR4 with many infectious and inflammatory diseases emphasizes its therapeutic importance. Subtle variations in the structure of lipid A profoundly affect activity, leading to unpredictable changes in TLR4 immune responses. To dissect the mechanisms of recognition and signaling, an extensive, explicitly-solvated, all-atom molecular dynamics simulation study has been performed for the isolated lipid-binding MD-2 co-receptor, and for the entire active, multimeric TLR4/MD-2 receptor complex, in the presence of a range of bound lipid A analogues, including several synthetic immunomodulatory mimetics undergoing clinical trials. We show that concerted large-scale conformational changes in MD-2 control the signaling status of the receptor complex, responding to variations in ligand acyl tail composition and headgroup phosphorylation status. Moreover, the co-receptor is part of a lipid-recognition superfamily, comprising immunoglobulin-like proteins involved in lipid signaling, transfer, and metabolism. Thus, we also report simulations of a wide range of these proteins, with or without ligands (including polyethers, fatty acids, single-chain lipids, and sterols), which reveal ligand-responsive conformational dynamics comparable to TLR4/MD-2. Therefore, a shared mechanism is identified in these distantly related family members, explaining variations in available structures obtained using different experimental approaches, and suggesting a molecular basis for allergic mimicry in dust-mite allergen lipid-binding proteins.

3376-Pos Board B237
A Common Model for Cytokine Receptor Activation: Combined Scissor-Like Rotation and Self-Rotation of Receptor Dimer Induced by Class I Cytokine
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The precise mechanism by which the binding of a class I cytokine to the extracellular domain of its corresponding receptor transmits a signal through the cell membrane remains unclear. Receptor activation involves a cytokine-receptor complex with a 1:2 stoichiometry. Previously we used our transient-complex theory to calculate the rate constant of the initial cytokine-receptor binding to form a 1:1 complex. Here we computed the binding pathway leading to the 1:2 activation complex. Three cytokine systems (growth hormone, erythropoietin, and prolactin) were studied, and the focus was on the binding of the extracellular domain of the second receptor molecule after forming the 1:1 complex. According to the transient-complex theory, translational and rotation diffusion of the binding entities bring them together to form a transient complex, which has near-native relative separation and orientation but not the short-range specific native interactions. Subsequently conformational rearrangement leads to the formation of the native complex. We found that the